Decabromodiphenylether in Human Whole Blood and Serum

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Introduction

Previous studies have reported detectable levels of decabromodiphenylether (decaBDE or BDE209) in human serum (*e.g.* Sjodin *et al.* 1999, Thomsen *et al.* 2007, Thuresson *et al.* 2005). A new study has been initiated to monitor decaBDE over a 10-year period in human blood in order to support the EU risk assessment of this chemical. DecaBDE is a large, highly lipophilic molecule with a log K_{ow} of ca. 9 – properties that do not allow free decaBDE to be detected in the aqueous phase. Any decaBDE present in human blood (a complex matrix) will be almost completely absorbed in or adsorbed to the lipid and protein fractions in the blood. In most studies of internal concentrations of organic pollutants in humans, serum (or sometimes plasma) is sampled for analysis. However, cellular material accounts for between 36 and 50% of the weight of whole blood, and whole blood contains clotting factor proteins that are absent in serum. These are likely to be important phases to which decaBDE partitions. Due to the unusually bulky structure of decaBDE compared to other common organic contaminants, its partitioning behaviour and detection issues in whole blood vs. serum cannot easily be predicted *a priori*.

The aim of the present study was therefore to determine the more appropriate phase in which to monitor decaBDE, whole blood or serum. Blood samples were collected to analyse decaBDE in i) whole blood, ii) serum and iii) the cellular fraction in order to assess the distribution of decaBDE over the different fractions of blood. The analysis of serum was performed by two different laboratories, the Institute for Environmental Studies (IVM) in Amsterdam and the Norwegian Institute of Public Health (NIPH) in Oslo, in order to compare results from independent analyses.

Materials and Methods

Sampling and serum preparation. Venous blood was collected from 10 healthy donors (employed in the Netherlands as laboratory and computer technicians and office workers) into preweighed BD Vacutainers® made of medical grade polyethylene. A minimum of 100 ml was collected from each donor, drawing alternately into 10 ml tubes (K_2EDTA coated, for whole blood samples) and 11 ml tubes (dry, empty tubes for serum). Whole blood samples were inverted 10 times immediately after drawing to mix the EDTA with the sample. All samples were kept in the dark from the time the samples were taken to avoid photodegradation of decaBDE. Care was taken to avoid typical sources of decaBDE contamination (*e.g.* dust, contaminated glassware). Samples designated for serum separation were allowed to clot for 30-45 minutes at room temperature and if centrifugation was not possible at that time, samples were stored at 4 ºC (maximum of 24 h) until centrifugation. Coagulated samples were centrifuged for 10 minutes at 1800 g. The serum was pipetted with a glass pipette and all serum was pooled per donor. Cell fractions (pellet) were stored in the original tubes. The masses of serum and cell fractions were

noted. All blood, serum and cell fraction samples were stored at –20 ºC until analysis. Sample vial blanks (n=3) of each type were reserved for checking decaBDE background. Subsamples from each of the 10 donors were couriered on dry ice to the NIPH in Oslo for analysis and an interlaboratory comparison of concentrations measured in serum.

Extraction, cleanup and analysis. At the IVM laboratory, decaBDE was extracted from all matrices (10 g) by acidification of the sample followed by liquid-liquid extraction. In each series, one blank (10 g analytical grade water), one reference material (NIST-SRM 1589a, certified for PBDEs but not for decaBDE) and one sample in duplicate were analysed. Samples were spiked with ^{13}C-decaBDE as internal standard. To acidify the samples and denature proteins, 6M HCl was added. After adding isopropanol, the sample was extracted twice with 12 ml hexane:methyl tertiary butylether (MTBE) (1:1 v/v). KCl was added, followed by centrifugation and extraction of the supernatant two more times with hexane:MTBE. The extract was then evaporated until almost dry before hexane was added. Concentrated H_2SO_4 was added, and the organic phase removed after centrifugation, followed by another extraction of the acid phase with hexane. The extract was evaporated to 1 ml under a mild stream of N_2.

The extracts were cleaned using a sulphuric acid-silica column followed by a neutral silica column. The extracts were first brought onto columns containing 0.1 g activated SiO_2 and 1 g activated SiO_2/H_2SO_4 (33% w/w). The columns were eluted with 30% dichloromethane:hexane (3:7 v/v). The extracts were collected and evaporated to ca. 1 ml under a mild stream of N_2. The extract was eluted with isooctane followed by isooctane:diethylether (85:15 v/v) on a column containing 1.8 g activated SiO_2/H_20 (1.5% w/w). The cleaned extracts were analysed by gas chromatography with electron capture negative chemical ionisation mass spectrometry detection (GC/ENCI-MS), based on the specific conditions for decaBDE analysis as described in de Boer *et al*. (2001). An extra short GC column (2 m) with 0.1 mm internal diameter, and a film thickness of 0.1 μm was used with He as carrier gas.

At the NIPH, ^{13}C-decaBDE was added to the serum samples as internal standard and extracted according to a recently published method using automated solid phase extraction (Thomsen *et al*. 2007). DecaBDE was determined with GC/ENCI-MS after chromatography on a 15 m column with 0.25 mm internal diameter and a film thickness of 0.10 μm.

Results and Discussion

DecaBDE was detected in samples from all 10 donors in both serum and whole blood (Fig. 1). Whole blood concentrations (mean 5.6 pg/g ww, s.d. 3.5, min. 2.3, max. 14 pg/g ww) measured by IVM were in the same range as serum concentrations, (mean 7.7 pg/g ww, s.d. 3.8, min. 3.7, max. 14.7 pg/g ww). DecaBDE was detectable in both matrices with the same frequency. Thuresson *et al*. (2005) reported decaBDE levels between 3.7 and 112 pg/g ww in serum of non-occupationally exposed referent workers, and the levels found in the present study were at the lower end of this range.

In the 10 donors, serum accounted for 42% of the wet weight of the whole blood on average (min. 38%, max 45%). If decaBDE were concentrated in serum alone, then we would expect to see concentrations in serum a factor of 2.4 higher than in whole blood. The ratio of DBDE in serum:whole blood varied from 0.93:1 to 2.5:1; on average this factor was 1.5 (s.d. 0.5), indicating that whole blood constituents not present in serum contain decaBDE as well.

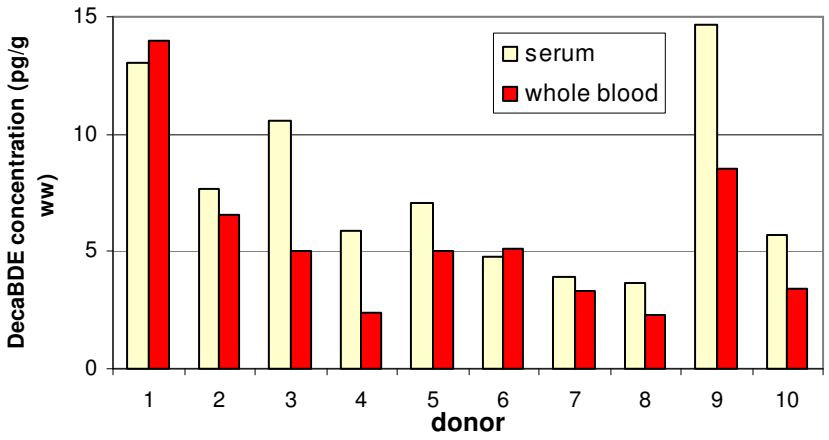

Figure 1. DecaBDE concentrations in serum and whole blood (sampled at the same time), from 10 individuals (data from IVM).

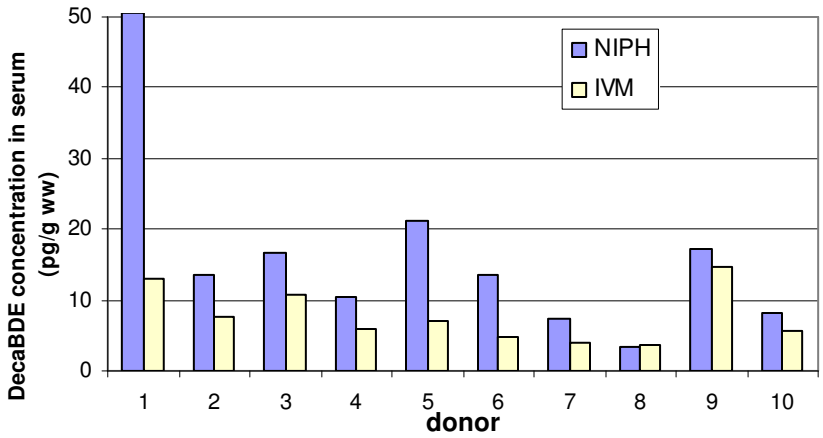

Figure 2. Serum concentrations of decaBDE measured by NIPH and IVM in subsamples of serum taken from 10 donors.

In six of the donors (Nos. 1, 3, 5, 6, and 9), we also analysed decaBDE in the pellet left after serum centrifugation. Here, decaBDE was detected in only one sample ('donor 9') at a level of 0.51 pg/g ww. The lack of detection is probably due to the difficulties of extracting decaBDE from a blood sample that had been allowed to clot (which is necessary according to the definition of preparation of serum). It should be mentioned that the pellet was a very difficult matrix to analyse and only interesting from the point of view of trying to increase understanding decaBDE partitioning in human blood, not as a matrix for monitoring. Whole blood samples did not have this clotting problem because it was possible to use EDTA-coated tubes to keep the blood sample fluid and reduce the risk of occlusion of decaBDE in the matrix.

For the interlaboratory comparison of serum concentrations, NIPH analysed decaBDE in serum and detected it in all 10 donors as well (Fig. 2). All concentrations measured were corrected for blanks by both laboratories. The levels were quite comparable to IVM data in most cases (particularly considering the low levels being measured), however particularly donor 1 and to a lesser extent donors 5 and 6 showed the largest discrepancies. Excluding these data, the two laboratories produced data that were within factor 1.5 of each other. Including all data, serum concentrations determined by NIPH using SPE were on average a factor of 2 higher than the results obtained by IVM. The mean of the serum levels measured by IVM was 7.7 pg/g ww, (s.d. 3.8, min. 3.7, max. 14.7 pg/g ww) compared to a mean of 16.2 (s.d. 13.2, min. 3.4, max. 50.7 pg/g ww) measured by NIPH, including all data. NIPH reported procedural blanks for the serum series for this study that were about factor 2 lower than normally observed, and suggested that the reported levels in serum would be slightly lower if they were corrected for the mean blank level.

Whole blood extracts produced chromatograms that were equally free of interference as those for serum using the IVM liquid-liquid extraction method. The LOD for the IVM method was 1.8 pg/g ww for whole blood and for 0.9 pg/g ww serum.

As this and other studies have demonstrated, the detection decaBDE in human blood requires LODs in the pg/g range. DecaBDE analysis at these levels presents the challenge to laboratories to avoid contamination of samples and keep the analytical and sampling blanks low. In any monitoring study, the sampling steps are equally critical to the production of reliable, quality data as the analytical steps. Since blood samples will be taken by health care professionals at various locations across Europe as part of the monitoring study, risks of decaBDE contamination would be reduced by using whole blood samples. The reason is, vacuvials for whole blood samples need not be centrifuged and opened for separation of serum from the pellet. The commonly used serum separator tubes cannot be used for sampling for decaBDE analysis due to gels present in the tubes, which can absorb the analyte (Berk *et al*. 2006).

The results reported above also suggest that a significant fraction of the decaBDE to be monitored partitions to components of whole blood that are absent in serum. Another practical advantage of whole blood sampling is that about 2.5 times less blood needs to be collected from the donor for the preparation of the sample (since serum makes up about 40% of the whole blood sample).

The extremely high lipophilicity of decaBDE, the variation in the ratio of decaBDE concentrations in whole blood and serum in the donors analysed, and in addition, a series of analytical and practical considerations lead to the recommendation to monitor in whole blood for the duration of the monitoring study.

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